



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> :	A3	(11) International Publication Number:	WO 95/34668
C12N 15/83, 15/11, 15/53, 15/52, 5/10, A01H 5/00		(43) International Publication Date:	21 December 1995 (21.12.95)
(21) International Application Number:	PCT/US95/06741	(81) Designated States:	AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KE, KG, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).
(22) International Filing Date:	26 May 1995 (26.05.95)	(20) Priority Data:	260,546 16 June 1994 (16.06.94) US
(71) Applicant: BIOSOURCE TECHNOLOGIES, INC. [US/US]; 3333 Vaca Valley Parkway, Vacaville, CA 95688 (US).		(72) Inventors: KUMAGAI, Monto, H.; 1330 Brown Drive, Davis, CA 95616 (US). DELLA-CIOPPA, Guy, R.; 814 Derry Circle, Vacaville, CA 95688 (US). DONSON, Jonathan; 717 Alvarado Avenue #233, Davis, CA 95616 (US). HARVEY, Damon, A.; 409 Eagle Lane, Vacaville, CA 95687 (US).	(74) Agents: HALLUN, Albert, P. et al.; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).

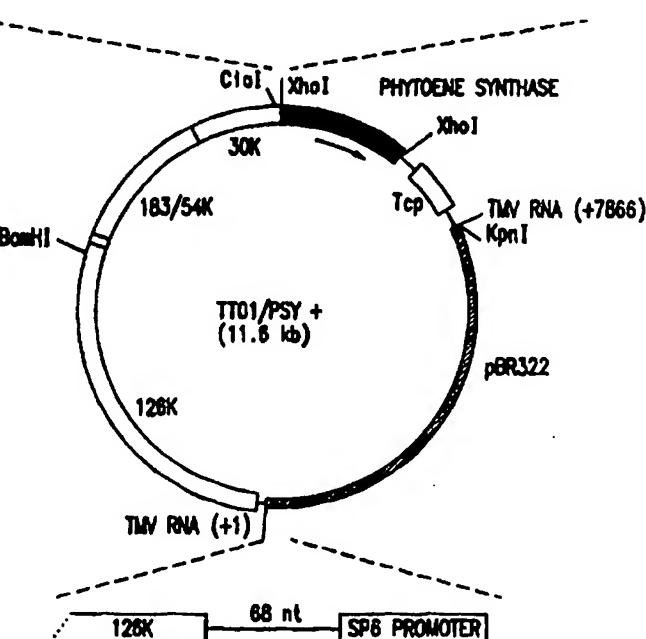
(54) Title: THE CYTOPLASMIC INHIBITION OF GENE EXPRESSION

(57) Abstract

One aspect of the invention is to provide novel genetic constructions for the expression of inhibitory RNA in the cytoplasm of eukaryotic cells. The inhibitory RNA may be an anti-sense RNA or a co-suppressor RNA. The genetic constructions of the invention are capable of replicating in the cytoplasm of a eukaryotic cell and comprise a promoter region in functional combination with an encoding polynucleotide. The genetic constructions may be designed so as to replicate in the cytoplasm of plant cells, yeast cells, and mammalian cells. When the eukaryotic cell of interest is a plant cell, the genetic construction is preferably derived from a plant RNA virus. Plant RNA virus derived genetic constructions may employ a plant virus subgenomic promoter, including subgenomic promoters from tobamoviruses in functional combination with the RNA encoding region. Another aspect of the invention is to provide cells comprising the genetic constructions of the invention and organism comprising a plurality of such cells. Another aspect of the invention is to provide methods of reducing the expression of a gene of interest in eukaryotic cells, i.e., methods of producing eukaryotic cells exhibiting reduced levels of expression of a gene of interest. The methods of the invention comprise the step of transfecting a cell with a genetic construction of the invention in which the RNA encoding region is specific for the gene of interest. Another aspect of the invention is to provide plant cells that produce elevated levels of the carotenoid phytoene. The elevated levels of phytoene are achieved by inhibiting the expression at the enzyme phytoene desaturase using the vectors of the invention.

.tsp XbaI Start codon  
CTT TTA TAA CCG CTC GGT TTT TAA ATG TCT GTT CCC TGG TTA TCG GTT GTT TCT CCT TGT GAC  
Met Ser Val Ala Leu Lys Trp Val Val Ser Pro Cys Asp

TRANSIT PEPTIDE OF PHYTOENE SYNTHASE  
GTC TCA AAT CGG ACA AGT TTC AGC GAA TCA GTC CGG GAG CGA ANC CGT  
Val Ser Asn Gly Thr Ser Phe Met Glu Ser Val Arg Glu Gly Asn Arg



Best Available Copy

***FOR THE PURPOSES OF INFORMATION ONLY***

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

**AMENDED CLAIMS**

[received by the International Bureau on 29 February 1996 (29.02.96);  
original claims 1-38 replaced by amended claims 1-44 (4 pages)]

desaturase is arranged in the antisense direction in relation to the promoter region which is in functional combination with the phytoene desaturase.

- 5        10. A vector according to Claim 8 wherein the target gene inhibitor RNA encoding polynucleotide is a phytoene synthase, wherein the phytoene synthase is arranged in the antisense direction in relation to the promoter region which is in functional combination with the phytoene synthase.
- 10      11. A method of producing a eukaryotic cell having reduced expression of a gene of interest, the method comprising the steps of transfecting a cell with a genetic vector according to Claim 1, wherein the anti-sense RNA encoded by the anti-sense RNA encoding polynucleotide is specific for the gene of  
15      interest.
12. A method according to Claim 11, wherein the eukaryotic cell is selected from the group consisting of plant cells, yeast cells, and mammalian cells.
- 20      13. A method according to Claim 12, wherein the eukaryotic cell is a plant cell.
- 25      14. A method according to Claim 13, wherein the vector is derived from a plant RNA virus.
15. A method according to Claim 14, wherein the vector is derived from a single stranded RNA plant virus.
- 30      16. A method according to Claim 15, wherein the promoter is a plant viral RNA subgenomic promoter.

35

17. A method according to Claim 15, further comprising a second subgenomic promoter operably linked to a viral coat protein encoding polynucleotide.
18. A method according to Claim 15, wherein at least one of the subgenomic promoters is derived from a tobarnovirus.
19. A method of producing a eukaryotic cell having reduced expression of a gene of interest, the method comprising the steps of transfecting a cell with a genetic vector according to Claim 9, and then growing the transfected cell under conditions suitable for growth of the vector.
20. A method of producing a eukaryotic cell having reduced expression of a gene of interest, the method comprising the steps of transfecting a cell with a genetic vector according to Claim 10, and then growing the transfected cell under conditions suitable for growth of the vector.
21. A eukaryotic cell produced by the method of Claim 11.
22. A eukaryotic cell according to Claim 21, wherein the eukaryotic cell is selected from the group consisting of plant cells, yeast cells, and mammalian cells.
23. A eukaryotic cell according to Claim 22, wherein the eukaryotic cell is a plant cell.
24. A eukaryotic cell according to Claim 23, wherein the vector is derived from a plant RNA virus.

Best Available Copy

25. A eukaryotic cell according to Claim 24, wherein the vector is derived from a single stranded RNA plant virus.
- 5 26. A eukaryotic cell according to Claim 24, wherein the promoter is a plant viral RNA subgenomic promoter.
- 10 27. A eukaryotic cell according to Claim 24, further comprising a second subgenomic promoter operably linked to a viral coat protein encoding polynucleotide.
- 15 28. A eukaryotic cell according to Claim 24, wherein at least one of the subgenomic promoters is derived from a tobamovirus.
- 20 29. A plant comprising a plurality of cells according to Claim 23.
30. A plant according to Claim 29, wherein the vector is derived from a plant RNA virus.
- 25 31. A plant according to Claim 30, wherein the vector is derived from a single stranded RNA plant virus.
- 30 32. A plant according to Claim 31, wherein the promoter is a plant viral RNA subgenomic promoter.
- 35 33. A plant according to Claim 31, further comprising a second subgenomic promoter operably linked to a viral coat protein encoding polynucleotide.
34. A plant according to Claim 31, wherein at least one of the subgenomic promoters is derived from a tobamovirus.

Best Available Copy

35. A eukaryotic cell comprising a vector according to Claim 1.
36. A eukaryotic cell according to Claim 35, wherein the eukaryotic cell is selected from the group consisting of plant cells, yeast cells, and mammalian cells.
37. A eukaryotic cell according to Claim 36, wherein the eukaryotic cell is a plant cell.
- 10 38. A eukaryotic cell according to Claim 37, wherein the vector is derived from a plant RNA virus.
- 15 39. A eukaryotic cell according to Claim 38, wherein the vector is derived from a single stranded RNA plant virus.
- 20 40. A eukaryotic cell according to Claim 39, wherein the promoter is a plant viral RNA subgenomic promoter.
- 25 41. A eukaryotic cell according to Claim 39, further comprising a second subgenomic promoter operably linked to a viral coat protein encoding polynucleotide.
42. A eukaryotic cell according to Claim 39, wherein at least one of the subgenomic promoters is derived from a tobamovirus.
- 30 43. A eukaryotic cell according to Claim 37 comprising a vector according to claim 9.
44. A method according to Claim 37 comprising a vector according to  
35 claim 10.

Post Available Copy